Serial Number: 09/458,779

Filing Date: December 10, 1999

Title: SEQUENCE AND METHOD FOR INCREASING PROTEIN EXPRESSION IN CELLULAR EXPRESSION SYSTEMS

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[Amended] The polynucleotide of claim [1] 3, further comprising a sequence encoding a heterologous target protein.

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[Amended] The polynucleotide of claim 2, wherein the yeast [protein] is [from] a Saccharomyces cerevisiae, Schizosaccharomyces pombe, Yarrowia lipolytica, Pichia pastoris, Hansenula polymorpha, or Kluyveromyces lactis.

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- 14. [Amended] [The expression vector of claim 13] A polynucleotide expression vector comprising a polynucleotide encoding a functional Vff2p, [where in] wherein the Vff2p comprises SEQ ID NO:2, or a variant thereof, and wherein the Vff2p is a yeast protein involved in the secretory pathway and/or involved in the required cellular machinery for membrane fusion.
- 15. [Amended] The expression vector of claim [18] 14, comprising SEQ ID NO:1, or a variant thereof.
- 16. [Amended] The expression vector of claim [13] 14, wherein the protein is about 32 kD.
- 17. [Amended] The expression vector of claim [13] 14, further comprising a promoter sequence operatively linked to the sequence encoding the Vff2p.
- 19. [Amended] The expression vector of claim [13] 14, further comprising a sequence encoding a heterologous target protein.
- 23.

[Amended] The expression vector of claim 22, wherein the [protein] <u>yeast is [from]</u>
Saccharomyces cerevisiae, Schizosaccharomyces pombe, Yarrowia lipolytica, Pichia
pastoris, Hansenula polymorpha, or Kluyveromyces lactis.

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- 25. [Amended] [The host cell of claim 24] A recombinant host cell comprising a yeast cell genetically altered to express a protein encoded by a polynucleotide sequence encoding a functional Vff2p, [where in] wherein the Vff2p comprises SEQ ID NO:2, or a variant thereof, and wherein the Vff2p is a yeast protein involved in the secretory pathway and/or involved in the required cellular machinery for membrane fusion.
- 26. [Amended] The host cell of claim [24] <u>25</u>, comprising SEQ ID NO:1, or a variant thereof.

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- 29. [Amended] The host cell of claim [28] 25, wherein the yeast cell is a Saccharomyces cerevisiae, Schizosaccharomyces pombe, Yarrowia lipolytica, Pichia pastoris, Hansenula polymorpha, or Kluyveromyces lastis cell.
- 30. [Amended] The host cell of claim [28] 25, wherein the host cell lacks a functional protein involved in the secretory pathway and/or involved in the required cellular machinery for membrane fusion, other than Vff2p.

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- 31. [Amended] A method for increasing [protein production in] <u>cell growth of</u> a host cell, comprising introducing Vff2p [to] <u>into</u> the cell and culturing the cell, <u>wherein the Vff2p</u> has at least 40% homology to SEQ ID NO:2.
- 32. [Amended] The method for increasing [protein production in] <u>cell growth of</u> a cell according to claim 31, wherein [a polynucleotide encoding Vff2p is introduced into the host cell and thereafter, culturing] the host cell <u>is cultured</u> under conditions effective to allow expression of the encoded Vff2p.
- 33. [Amended] A method for increasing protein secretion from a host cell, comprising introducing Vff2p [to] into the cell and culturing the cell, wherein the Vff2p has at least 40% homology to SEQ ID NO:2.

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34. [Amended] The method for increasing protein secretion from a cell according to claim 33, wherein [a polynucleotide encoding Wf2p is introduced into the host cell and thereafter, culturing] the host cell is cultured under conditions effective to allow expression of the encoded Vff2p.

36. [Amended] An isolated functional vesicular fusion factor 2 protein [The protein of claim 35 having an amino acid sequence] comprising SEQ ID NO:2, or a variant thereof, and wherein the Vff2p is a yeast protein involved in the secretory pathway and/or involved in the required cellular machinery for membrane fusion.

37. [Amended] A method of selecting for a yeast secretory mutant cell containing a polynucleotide sequence encoding a Vff2p[, or a structural homolog of Vff2p,] operably linked to a promoter, wherein the Vff2p comprises SEQ ID NO:2, or a variant thereof, the method comprising growing the secretory mutant cell at a restrictive temperature of about 32-37°C, wherein the restrictive temperature selectively favors mutant cell growth.

Please add the following new claims:

43. [New] The polynucleotide of claim 3, wherein the Vff2p comprises SEQ ID NO:2.

[New] The protein of claim 25, wherein the yeast is S. cerevisiae.

- 45. [New] The protein of claim 36, wherein the protein is from S. cerevisiae.
- 46. [New] The method of claim 37, wherein the yeast cell is a Saccharomyces cerevisiae, Schizosaccharomyces pombe, Yarrowia lipolytica, Pichia pastoris, Hansenula polymorpha, or Kluyveromyces lactis cell.

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